Folate Antagonists. 10. Synthesis and Antimalarial Effects of 6-[[(Aryl and aralkyl)amino]methyl]-2,4-pteridinediamines and -pteridinediamine 8-Oxides[1-3]

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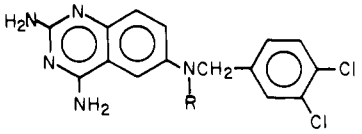
Various 6-[[(aryl and aralkyl)amino]methyl]-2,4-pteridinediamines and their 8-oxides have been synthesized for antimalarial evaluation. Condensation of 3-amino-6-(bromomethyl)-2-pyrazinecarbonitrile 4-oxide (V) with the appropriately substituted amine afforded a series of 3-amino-6-[[(aryl and aralkyl)amino]methyl]-2-pyrazinecarbonitrile 4-oxides VI. Deoxygenation gave the corresponding pyrazines VII. Cyclization of VI and VII with guanidine then produced the desired 6-(aminomethyl)-2,4-pteridinediamine *N*-oxides VIII and pteridinediamines IX, respectively. Formylation of 6-[[(3,4-dichlorophenyl)amino]methyl]-2,4-pteridinediamine gave *N*-[(2,4-diamino-6-pteridinyl)-methyl]-*N*-(3,4-dichlorophenyl)formamide. The *N*-oxides VIII did not exhibit significant activity against *Plasmodium berghei* infections in mice. Activity among the 2,4-pteridinediamines IX was generally poor with the exception of the 3,4,5-trimethoxyphenyl and 1-naphthalenyl analogues which showed strong suppressive activity at doses ranging from 80 to 640 mg/kg. Furthermore, several of the 2,4-pteridinediamines exhibited potent prophylactic activity against *Plasmodium gallinaceum* infections in the chick and also showed strong antibacterial action against *Streptococcus faecalis* and *Staphylococcus aureus*.

A broad range of nonclassical pteridine-2,4-diamine antifolates, exemplified by 6,7-diphenyl-2,4-pteridinediamine, 6,7-bis(1-methylethyl)-2,4-pteridinediamine, and 6-(2-methylphenyl)-2,4,7-pteridinetriamine, possesses an appreciable antimalarial effect against *Plasmodium gallinaceum* and *Plasmodium berghei*.[4] Unfortunately, cross-resistance with cycloguanil and pyrimethamine is prevalent among this class of antimetabolites.[4]

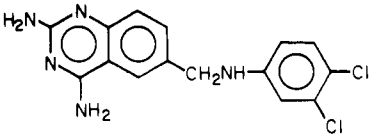
Numerous 6-[(phenylmethyl)amino]-2,4-quinazolinediamines, exemplified by 6-[[(3,4-dichlorophenyl)-methyl]amino]-2,4-quinazolinediamine (Ia),[5] 6-[[(3,4-di-



Ia, R = H
b, R = CH_3
c, R = CHO

chlorophenyl)methyl]methylamino]-2,4-quinazolinediamine (Ib),[6] and *N*-(2,4-diamino-6-quinazolinyl)-*N*-[(3,4-dichlorophenyl)methyl]formamide (Ic),[7] exhibit strong antimalarial and antibacterial effects.[5-7]

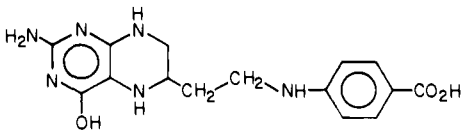
A series of 6-[(phenylamino)methyl]-2,4-quinazolinediamines[7] represented by II was considerably more potent



II

an antimalarial than Ia and, in addition, appeared to have only a small degree of cross-resistance against a cycloguanil-resistant strain of *P. berghei*.[8]

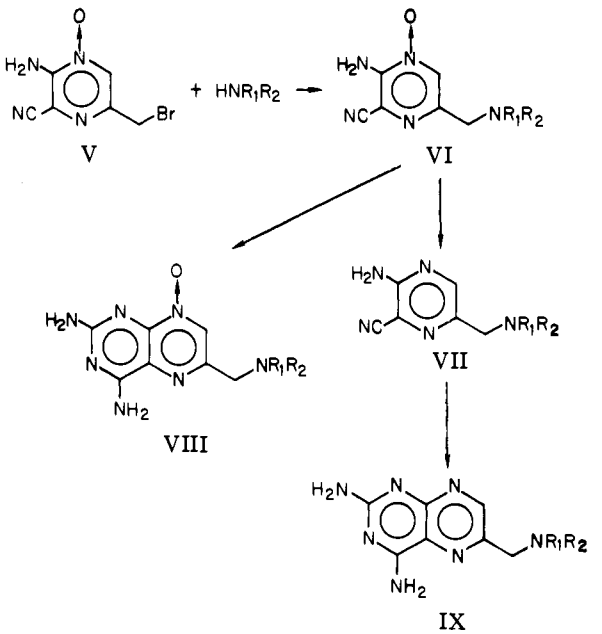
Furthermore, tetrahydrohomopteroic acid (III), an



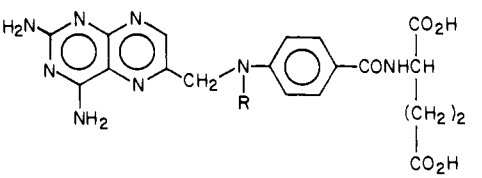
III

analogue of folic acid containing the pteridine ring but not the glutamic acid side chain, has been reported to be effective against both pyrimethamine sensitive and resistant strains of *Plasmodium cynomolgi* in rhesus monkeys.[9]

Scheme I



It seemed pertinent, therefore, to proceed with the preparation of various 6-[[(aryl and aralkyl)amino]-methyl]-2,4-pteridinediamines as nonclassical analogues of aminopterin (IV, R = H) and methotrexate (IV, R = CH_3).



IV

Chemistry. Recent reports by Taylor et al.[10] have described an efficient synthesis of several 2,4-pteridinediamines proceeding via a series of 3-amino-2-pyrazinecarbonitrile 4-oxides. Two preliminary accounts demonstrating the use of this route for the preparation of some pteroic acid and methotrexate relatives have recently appeared,[11] and Professor Taylor has prepared one of our compounds (**39**) in a recent manuscript delineating his

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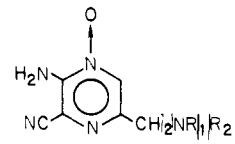
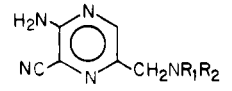
Table I. 3-Amino-6-[[(aryl and aralkyl)amino]methyl]-2-pyrazinecarbonitrile 4-Oxides

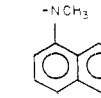


Compd no.	-NR_1R_2	Mp, °C	Yield purified, %	Crystn solvent	Formula	Analyses
1	-NH-C_6H_3-3,4-Cl_2	225-227 dec	22	EtOH	$C_{12}H_9Cl_2N_5O$	C, H, N
2	-N(CH_3)-C_6H_4-4-Cl	140-143	36	2-PrOH	$C_{13}H_{12}ClN_5O$	C, H, N
3	-N(CH_3)-C_6H_3-3,4-Cl_2	173-175	62	2-PrOH	$C_{13}H_{11}Cl_2N_5O$	C, H, N
4	-N(CH_3)-C_6H_2-3,4,5-(OCH_3)$_3$	167-170	43	EtOH	$C_{16}H_{19}N_5O_4$	C, H, N
5	-N(C_2H_5)-C_6H_4-4-Cl	137-140	48	EtOH-2-PrOH	$C_{14}H_{14}ClN_5O$	C, H, N
6	-N(C_2H_5)-C_6H_4-4-OCH_3	103-105	71	2-PrOH	$C_{15}H_{17}N_5O_2$	C, H, N
7	-N[(CH_2)$_2$$CH_3$]-$C_6H_3$-3,4-$Cl_2$	128-130	56	2-PrOH	$C_{15}H_{15}Cl_2N_5O$	C, H, N
8	-N[$CH(CH_3)_2$]-C_6H_4-4-Cl	*a*	72	*a*	$C_{15}H_{16}ClN_5O$	
9	-N[$CH(CH_3)_2$]-C_6H_3-3,4-Cl_2	*a*	64	*a*	$C_{15}H_{15}Cl_2N_5O$	
10		149-152[b]	40	EtOH	$C_{14}H_{13}N_5O$	C, H, N
11		175-177	52	2-PrOH	$C_{15}H_{15}N_5O$	C, H, N
12	c-NC_5H_9-2-C_6H_5	*a*	51	*a*	$C_{17}H_{19}N_5O$	
13	c-NC_5H_9-2-$CH_2C_6H_5$	*a*	65	*a*	$C_{18}H_{21}N_5O$	
14		113-116	55	EtOH	$C_{17}H_{15}N_5O$	C, H, N

[a] These compounds were purified by column chromatography and spectrally characterized but were not obtained as crystalline solids. [b] After softening and partial melting at 135 °C; considered to be due to the presence of two crystal forms.

Table II. 3-Amino-6-[[(aryl and aralkyl)amino]methyl]-2-pyrazinecarbonitriles



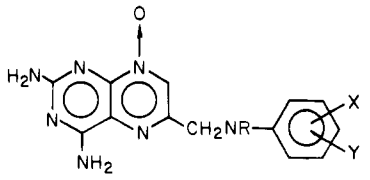
Compd no.	-NR_1R_2	Mp, °C	Yield purified, %	Crystn solvent	Formula	Analyses
15	-NH-C_6H_3-3,4-Cl_2	188-192	78	EtOH	$C_{12}H_9Cl_2N_5$	C, N; H[a]
16	-N(CH_3)-C_6H_4-4-Cl	97-100	71	2-PrOH	$C_{13}H_{12}ClN_5$	C, H, N
17	-N(CH_3)-C_6H_3-3,4-Cl_2	158-160	66	EtOH	$C_{13}H_{11}Cl_2N_5$	C, H, N
18	-N(CH_3)-C_6H_2-3,4,5-(OCH_3)$_3$	147-149	80	EtOH	$C_{16}H_{19}N_5O_3$	C, H, N
19	-N(C_2H_5)-C_6H_4-4-Cl	108-110	73	2-PrOH	$C_{14}H_{14}ClN_5$	C, H, N
20	-N(C_2H_5)-C_6H_4-4-OCH_3	106-108	74	2-PrOH	$C_{15}H_{17}N_5O$	C, H, N
21	-N[(CH_2)$_2$$CH_3$]-$C_6H_3$-3,4-$Cl_2$	93-96	83	2-PrOH	$C_{15}H_{15}Cl_2N_5$	C, H, N
22	-N[$CH(CH_3)_2$]-C_6H_4-4-Cl	107-110	71	2-PrOH	$C_{15}H_{16}ClN_5$	C, H, N
23	-N[$CH(CH_3)_2$]-C_6H_3-3,4-Cl_2	107-110	71	2-PrOH	$C_{15}H_{15}Cl_2N_5$	C, H, N
24		158-161	95	2-PrOH	$C_{14}H_{13}N_5$	C, H, N
25		161-163	90	2-PrOH	$C_{15}H_{15}N_5$	C, H, N
26	c-NC_5H_9-2-C_6H_5	145-147	71	2-PrOH	$C_{17}H_{19}N_5$	C, H, N
27	c-NC_5H_9-2-$CH_2C_6H_5$	143-146	82	2-PrOH	$C_{18}H_{21}N_5$	C, H, N
28		150-152	78	EtOH	$C_{17}H_{15}N_5$	C, H, N

[a] H: calcd, 3.08; found, 3.54.

method.[12] This route also proved effective for the preparation of the compounds reported herein (Scheme I).

Condensation of 3-amino-6-(bromomethyl)-2-pyrazinecarbonitrile 4-oxide (V)[13] with a variety of amines gave the 3-amino-6-(aminomethyl)-2-pyrazinecarbonitrile 4-

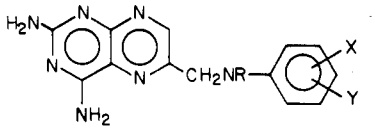
Table III. 6-[[(Aryl)amino]methyl]-2,4-pteridinediamine 8-Oxides



No.	R	X, Y	Mp, °C[a]	Yield purified, %[b]	Formula[c]
29	H	3,4-Cl_2	265	39	$C_{13}H_{11}Cl_2N_7O$
30	CH_3	4-Cl	275	44	$C_{14}H_{14}ClN_7O$
31	CH_3	3,4-Cl_2	283	74	$C_{14}H_{13}Cl_2N_7O$
32	CH_3	3,4,5-$(OCH_3)_3$	254	39	$C_{17}H_{21}N_7O_4$
33	C_2H_5	4-Cl	278	70	$C_{15}H_{16}ClN_7O$
34	C_2H_5	4-OCH_3	269	28	$C_{16}H_{19}N_7O_2 \cdot 0.5H_2O$
35	n-C_3H_7	3,4-Cl_2	265	47	$C_{16}H_{17}Cl_2N_7O$
36	$CH(CH_3)_2$	4-Cl	269	38	$C_{16}H_{18}ClN_7O$
37	$CH(CH_3)_2$	3,4-Cl_2	276	45	$C_{16}H_{17}Cl_2N_7O$
38	CH_3	2,3 	267	48	$C_{18}H_{17}N_7O$

[a] All compounds melted with decomposition. [b] All compounds were recrystallized from N,N-dimethylformamide. [c] Analytical results for C, H, and N were within ±0.4% of the theoretical values.

Table IV. 6-[[(Aryl)amino]methyl]-2,4-pteridinediamines



No.	R	X, Y	Mp, °C[a]	Yield purified, %[b]	Formula[c,d]
39	H	3,4-Cl_2	290-293	58	$C_{13}H_{11}Cl_2N_7 \cdot 0.3(CH_3)_2NCHO$
40	CH_3	4-Cl	270-272	42	$C_{14}H_{14}ClN_7$
41	CH_3	3,4-Cl_2	297-300	23	$C_{14}H_{13}Cl_2N_7$
42	CH_3	3,4,5-$(OCH_3)_3$	243-246	51	$C_{17}H_{21}N_7O_3$[c]
43	C_2H_5	4-Cl	287-289	60	$C_{15}H_{16}ClN_7$
44	C_2H_5	4-OCH_3	261	33	$C_{16}H_{19}N_7O$[c]
45	n-C_3H_7	3,4-Cl_2	258-260	56	$C_{16}H_{17}Cl_2N_7$
46	$CH(CH_3)_2$	4-Cl	283-285	61	$C_{16}H_{18}ClN_7 \cdot 0.5(CH_3)_2NCHO$
47	$CH(CH_3)_2$	3,4-Cl_2	290	62	$C_{16}H_{17}Cl_2N_7$
48	CH_3	2,3	274-275	49	$C_{18}N_{17}N_7$
49	CHO	3,4-Cl_2	268-270	81	$C_{14}H_{11}Cl_2N_7O$

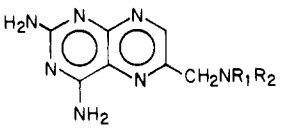
[a] See footnotes for Table III. [b] Compound 42 was recrystallized from N,N-dimethylformamide-H_2O, 47 from alcohol, 49 from alcohol-H_2O, and all others from N,N-dimethylformamide. [c] Analytical results for C, H, and N were within ±0.4% of the theoretical values except for 42 (C: calcd, 54.97; found, 54.52) and 44 (C: calcd, 59.06; found, 58.60). [d] The presence of solvation was confirmed by NMR.

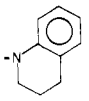
oxides VI (1-14, Table I) (22-72% yield). Deoxygenation of VI with triethyl phosphite gave the corresponding 3-amino-6-(aminomethyl)-2-pyrazinecarbonitriles VII (15-28, Table II) (66-95% yield). Cyclization of VI and VII with guanidine in refluxing ethanol then gave the desired 6-[[(aryl and aralkyl)amino]methyl]-2,4-pteridinediamine 8-oxides VIII (29-38, Table III, and 50, 52, 54, and 56, Table V) (28-74% yield) and the corresponding 2,4-pteridinediamines IX (39-48, Table IV, and 51, 53, 55, and 57, Table V) (23-62% yield), respectively. Formylation of 6-[[(3,4-dichlorophenyl)amino]methyl]-2,4-pteridinediamine (39) with formic acid gave N-[(2,4-diamino-6-pteridinyl)methyl]-N-(3,4-dichlorophenyl)formamide (49) in 81% yield.

The ultraviolet absorption maxima and the pK'_a values for a selected group of the 6-(aminomethyl)-2,4-pteridinediamines and the corresponding 8-oxides are listed in Table VI. The pK'_a values for the 2,4-pteridinediamine systems are approximately two units less than those of the corresponding 2,4-quinazolinediamines,[2-4] and those for the 2,4-pteridinediamine 8-oxides are even lower. Major variations occurred in those cases where the side chain contains an amine which is protonated at a higher pH than the pteridine ring system. For the 2,4-pteridinediamines listed in Tables IV and V, this occurred only when the benzene ring of the side chain was not attached directly to the nitrogen (57). In the case of the 8-oxides, 6-[[ethyl(4-methoxyphenyl)amino]methyl]-2,4-pteridinediamine 8-oxide (34) also showed this effect.

The 2,4-pteridinediamines are characterized in the neutral species by a low-intensity band at 375 nm and stronger absorption at ~260 nm. For the 8-oxides these maxima are shifted to 400 and ~268 nm, respectively. In acid the 2,4-pteridinediamines show peaks at 337 (shoulder at 350 nm) and ~250 nm, while the 8-oxides give maxima at 360 (shoulder at 375 nm) and near 260 nm.

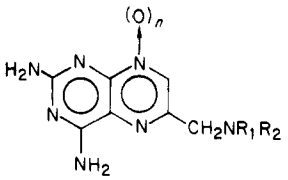
Table V. 6-[[(Aralkyl)amino]methyl]-2,4-pteridinediamines and 8-Oxides

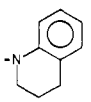


No.	NR_1R_2	Position 8	Mp, °C[a]	Yield purified, %[a]	Formula[a]
50		Oxide	262	65	$C_{15}H_{15}N_7O$
51			282–283	43	$C_{15}H_{15}N_7$
52		Oxide	257	57	$C_{16}H_{17}N_7O \cdot 0.9(CH_3)_2NCHO$
53			293	60	$C_{16}H_{17}N_7$
54	c-NC_5H_9-2-C_6H_5	Oxide	256	44	$C_{18}H_{21}N_7O$
55	c-NC_5H_9-2-C_6H_5		>300	59	$C_{18}H_{21}N_7 \cdot 0.2(CH_3)_2NCHO$
56	c-NC_5H_9-2-$CH_2C_6H_5$	Oxide	234	37	$C_{19}H_{23}N_7O \cdot (CH_3)_2NCHO$
57	c-NC_5H_9-2-$CH_2C_6H_5$		238–243	31	$C_{19}H_{23}N_7 \cdot 0.9(CH_3)_2NCHO$

[a] See footnotes in Table III.

Table VI. Ultraviolet and pK'_a Properties of Selected 6-[[(Aryl and aralkyl)amino]methyl]-2,4-pteridinediamines and the Corresponding 8-Oxides

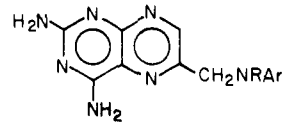


No.	$-NR_1R_2$	n	pK'_a[a] 6-Substituent	pK'_a[a] Pteridine	Ultraviolet absorption MeOH-KOH λ, nm	MeOH-KOH ε	MeOH-HCl[b] λ, nm	MeOH-HCl[b] ε
30	$-N(CH_3)$-C_6H_4-4-Cl	1	0.6	2.3	400	7800	360	7000
					268	40500	259	39500
44	$-N(CH_3)$-C_6H_4-4-Cl	0	1.1	5.1	375	7600	337	10200
					260	40000	250	23800
34	$-N(C_2H_5)$-C_6H_4-4-OCH_3	1	3.7	1.4	400	8400	362	7000
					268	38500	263	29800
48	$-N(C_2H_5)\cdot C_6H_4$-4-OCH_3	0	2.9	4.9	375	8400	337	10500
					257	35600	248	19300
37	$-N[CH(CH_3)_2]$-C_6H_3-3,4-Cl_2	1	0.3	2.0	400	8800	361	7900
					269	45600	260	42800
51	$-N[CH(CH_3)_2]\cdot C_6H_3$-3,4-$Cl_2$	0	0.8	4.9	377	8200	337	10500
					262	39200	249	23100
39		1	0.7	2.2	399	8900	360	8100
					268	43500	258	43500
53		0	1.0	5.0	375	7500	337	10500
					260	34000	248	21300
41	c-NC_5H_9-2-$CH_2C_6H_5$	1	6.8	1.5	397	6900	361	6200
					267	31300	261	25800
55	c-NC_5H_9-2-$CH_2C_6H_5$	0	7.3	4.0	373	7600	338	10300
					260	27000	248	18500

[a] The pK'_a values were determined spectrophotometrically in 50% methanol, with the exception of the higher value for 51 which was found by titration in 67% *N,N*-dimethylformamide. [b] Sufficient 5 N hydrochloric acid was added to form the monoprotonated pteridine ring.

Examination of the UV spectra of the 2,4-pteridinediamines and 8-oxides in acidic methanol prior to purification consistently showed absorption of variable magnitude at ~420 nm. This is apparently due to a by-product formed during the cyclization with guanidine. Similar absorption has been observed from the products obtained on cyclization of 3-amino-6-chloro-2-pyrazinecarbonitrile 4-oxide and *N*-[4-[[(5-amino-6-cyano-2-pyrazinyl)methyl]methylamino]benzoyl]glutamic acid diethyl ester but not from the cyclizations leading to

Table VII. Prophylactic Effects of 6-[[(Aryl and aralkyl)amino]methyl]-2,4-pteridinediamines against Sporozoite-Induced *P. gallinaceum* in Chicks



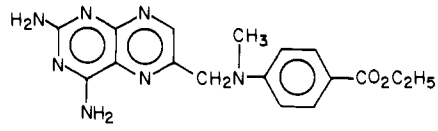
No.	ArNR	ΔMST or C after single sc dose[a] 480 (320)	120 (160)	60 (80)	30 (40)	15 (20)	7.5	3.75
44	$-N(C_2H_5)-C_6H_4-4-OCH_3$	(C5/5)		(C5/5)		(C5/5)		
45	$-N[(CH_2)_2CH_3]-C_6H_4-3,4-Cl_2$	C10/10	C20/20	C10/10	C18/20	C9/10	C9/10	C4/10
46	$-N[CH(CH_3)_2]-C_6H_4-4-Cl$	C10/10	C20/20	C10/10	C19/20	C10/10	C9/10	C6/10
53		C10/10	C20/20	C10/10	C20/20	C5/10	2.3	1.3
55	$c-NC_5H_9-2-C_6H_5$	0.3	0.2		0.1			
X	$-N(CH_3)-C_6H_4-4-CO_2C_2H_5$	C5/5	C5/5	C5/5	C4/5	C2/5		

[a] ΔMST is the mean survival time (days) of treated chicks (MSTT) minus the mean survival time (days) of control chicks (MSTC). In the present study, the MSTC ranged from 7.0 to 7.4 days. C designates the number of chicks surviving to 30-days postinfection and termed "cured"; data to establish parasitological cure based on subinoculation are unavailable. T indicates the number of deaths occurring within 48 h after infection which is attributed to drug action and is counted as toxic deaths. Control birds do not die before 48 h. Each entry at each dose level represents the results with a five-animal group.

2,4-quinazolinediamines.[5-7] Isolation and identification of these by-products are currently under investigation.

Suppressive Antimalarial Screening in Mice. The 6-[[(aryl and aralkyl)amino]methyl]-2,4-pteridinediamines IX as well as the *N*-oxide derivatives VIII were tested against a normal drug-sensitive strain of *P. berghei* in mice by the parenteral route.[14,15] The compounds were dissolved or suspended in sesame or peanut oil and were administered to mice in a single subcutaneous dose 72-h postinfection. Extension of the mean survival time of the treated mice is interpreted as evidence of antimalarial activity.[15] Compounds are arbitrarily considered to be "active" when they produce at least a 100% increase in the mean survival time of treated mice. Animals that survive to 60 days are considered "cured". The mean survival time of infected control mice in the present study ranged from 6.1 to 6.5 days.

None of the *N*-oxides exhibited significant activity against trophozoite-induced *P. berghei* infections in mice at doses up to 640 mg/kg. Activity among the 6-[[(aryl)amino]methyl]-2,4-pteridinediamines was also generally poor. However, exceptions were noted with the 3,4,5-trimethoxyphenyl analogue (**42**), which cured 5/5 mice at 320 mg/kg and showed strong suppressive activity (extension of survival time 8.5 days) at 160 mg/kg, and the 1-naphthalenyl analogue (**48**) which cured 5/5 mice at 320 mg/kg and showed suppressive activity (extension of survival time 5.4 days) down to 40 mg/kg. On the other hand, the prototype X prepared earlier by Modest and



X

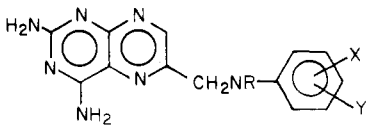
co-workers[16] lacked significant activity even at high doses. These results tend to support our working hypothesis that the introduction of certain nonpolar groups into the molecule should increase passive cellular transport and thus confer enhanced antimalarial properties.

Prophylactic Antimalarial Activity in Chicks.[14,17] Preliminary results with selected pteridines against sporozoite-induced *P. gallinaceum* infections in chicks are highly encouraging. White Leghorn cockerels were parasitized by the intrajugular injection of *P. gallinaceum* sporozoites. All control chicks died between 6 and 11 days postinfection. In the present study, the mean survival time of control animals ranged from 7.0 to 7.4 days. A drug is considered active if the mean survival time of treated chicks is at least twice as long as that of untreated control chicks or if any of the chicks survive to 30 days. The drugs were suspended in peanut oil and were administered subcutaneously in a single dose on the day of infection. Each compound was tested in groups of five chicks at one to six dose levels ranging from 3.75 to 480 mg/kg. Four of the five compounds (Table VII) tested to date are highly active and two of them, **45** and **46**, retain curative activity at 3.75 mg/kg, the lowest dose utilized. Both compounds were more active than the reference compound X.

Antimalarial Testing in Primates.[18] The 1-naphthalenyl analogue (**48**), when tested against a trophozoite-induced *P. cynomolgi* infection in a rhesus monkey at a single oral dose of 3.16 mg/kg and against a sporozoite-induced *P. cynomolgi* infection in a rhesus monkey at 10 mg/kg, resulted only in limited efficacy, and it is thus unlikely that further interest in this series will develop.

Antibacterial Studies. Each of the pteridinediamines and its *N*-oxide was tested in vitro against a spectrum of pathogenic bacteria including *Streptococcus faecalis* (MGH-2), normal (UC-76) and drug-resistant (S18713) *Staphylococcus aureus*, *Escherichia coli* (Vogel), and *Shigella sonnei* (C-10). A modification of the gradient plate procedure of Szybalski[19] and Webb and Washington[20] was employed throughout. The data are summarized in Table VIII. All the compounds were active against *S. faecalis* (MGH-2) at <0.25 μg/mL, most were active also against *S. aureus* (UC-76), but activity against the resistant form was only retained by five compounds. The pteridines were uniformly inactive against *E. coli* and *S. sonnei*. Generally, the *N*-oxides were less active than their corresponding members, usually retaining only a modest degree of activity against only *S. faecalis*. Of compounds **51**, **53**, **55**, and **57**, only **51** and **53** retained strong activity against *S. faecalis* and *S. aureus* (UC-76). Thus the pteridines generally exhibit the same spectrum of antibacterial activity and potency as the quinazolinediamines reported previously.[21,22]

Table VIII. In Vitro Antibacterial Effects of 6-[[(Aryl)amino]methyl]-2,4-pteridinediamines



No.	X, Y	R	Min inhibitory concn, μg/mL[a] *S.f.*[b] MGH-2	*S.a.*[c] UC-76	*S.a.*[c] S18713	*E.c.*[d] Vogel	*S.s.*[e] C-10
39	3,4-Cl_2	H	<0.25	<0.25	<0.25	>25	>25
40	4-Cl	CH_3	<0.25	1.5	2.0	>25	>25
41	3,4-Cl_2	CH_3	<0.25	<0.25	1.5	>25	>25
42	3,4,5-$(OCH_3)_3$	CH_3	<0.25	<0.25	<0.25	>25	>25
43	4-Cl	C_2H_5	<0.25	<0.25	1.5	>25	>25
44	4-OCH_3	C_2H_5	<0.25	<0.25	<0.25	>25	>25
45	3,4-Cl_2	$(CH_2)_2CH_3$	<0.25	<0.25	<0.25	>25	>25
46	4-Cl	$CH(CH_3)_2$	<0.25	<0.25	1.5	>25	>25
47	3,4-Cl_2	$CH(CH_3)_2$	<0.25	<0.25	<0.25	>25	>25
48	2,3	CH_3	<0.25	1.0	>2.5	>25	>25
49	3,4-Cl_2	CHO	<0.25	1.0	2.0	>25	>25

[a] Gradient plate test. [b] *S.f.* = *Streptococcus faecalis*. [c] *S.a.* = *Staphylococcus aureus*. [d] *E.c.* = *Escherichia coli*. [e] *S.s.* = *Shigella sonnei*.

Experimental Section[23]

3-Amino-6-[[(3,4-dichlorophenyl)propylamino]methyl]-2-pyrazinecarbonitrile 4-Oxide (7, Table I). A solution of 6.0 g (0.026 mol) of 3-amino-6-(bromomethyl)-2-pyrazinecarbonitrile 4-oxide[13] in 60 mL of $(CH_3)_2CO$ was added slowly to a stirred mixture of 3.6 g (0.026 mol) of K_2CO_3 and 4.9 g (0.024 mol) of 3,4-dichloro-*N*-propylbenzenamine in 60 mL of $(CH_3)_2CO$. After 2 h at room temperature, the mixture was filtered and concentrated on a rotary evaporator. The residue was dissolved in ethyl acetate–benzene (3:7) and chromatographed over 180 g of silica gel. Appropriate fractions were combined as determined by thin-layer chromatography [silica gel, ethyl acetate–benzene (3:7), R_f = ~0.3] and concentrated to an oil which crystallized on standing. Recrystallization from 2-propanol gave 5.1 g (56%) of yellow crystals.

Compounds 1–3, 5, 8–10, and 12–14 were obtained in the same manner. However, the residues obtained upon concentration of the eluates could not be crystallized in the case of 8, 9, 12, and 13. For compounds 4, 6, and 11 crystallization of the concentrate from the filtered reaction mixture was accomplished without the need of column chromatography.

3-Amino-6-[(2,3-dihydro-1*H*-indol-1-yl)methyl]-2-pyrazinecarbonitrile (24, Table II). A solution of 1.90 g (7.12 mmol) of 3-amino-6-[(2,3-dihydro-1*H*-indol-1-yl)methyl]-2-pyrazinecarbonitrile 4-oxide (10, Table I), 11.8 g (71.2 mmol) of triethyl phosphite, and 10 mL of DMF was stirred at 120 °C for 1 h and concentrated by rotary evaporation under a water aspirator. The residue was recrystallized from 2-propanol to yield 1.7 g (95%) of a pale yellow solid.

With the exception of 23, the remainder of the compounds from Table II were obtained in the same manner. In the case of 23, crystallization of the reaction concentrate could not be induced. It was, therefore, dissolved in ethyl acetate–benzene (3:7) and chromatographed over silica gel. Concentration of the appropriate fractions then gave a crystalline residue.

6-[[(3,4-Dichlorophenyl)(1-methylethyl)amino]methyl]-2,4-pteridinediamine 8-Oxide (37, Table III). A solution of 0.00434 mol of guanidine (freshly prepared from equimolar quantities of guanidine hydrochloride and sodium ethoxide) was added to 1.50 g (0.00426 mol) of 3-amino-6-[[(3,4-dichlorophenyl)(1-methylethyl)amino]methyl]-2-pyrazinecarbonitrile 4-oxide (9, Table I), and the mixture was stirred and heated under reflux for 2 h. The precipitate which formed was collected by filtration, washed with water, and recrystallized from DMF to give 0.75 g (45%) of yellow solid.

The remainder of the analogues in Table III (29–36 and 38) and Table V (50, 52, 54, and 56) were prepared in an analogous manner by cyclization of the appropriate 3-amino-2-pyrazinecarbonitrile 4-oxide (Table I) with guanidine in ethanol.

6-[[(3,4-Dichlorophenyl)amino]methyl]-2,4-pteridinediamine–0.3-*N*,*N*-Dimethylformamide (39, Table IV). A mixture of 0.51 g (0.0053 mol) of guanidine hydrochloride and a solution of 0.12 g (0.0052 mol) of Na in 20 mL of EtOH was warmed briefly, allowed to cool, and filtered. The filtrate was combined with 1.49 g (0.00507 mol) of 3-amino-6-[[(3,4-dichlorophenyl)amino]methyl]-2-pyrazinecarbonitrile (15, Table II) and enough EtOH to make the total volume 30 mL. The mixture was heated under reflux for 2 h, allowed to cool slightly, and filtered. The filter cake was washed with H_2O and acetone, recrystallized from DMF, and dried at 110 °C in vacuo (0.1 mm) to give 1.06 g (58%) of the title compound, mp 290–293 °C dec.

The remainder of the 6-(aminomethyl)-2,4-pteridinediamines listed in Table IV (40–48) and Table V (51, 53, 55, 57) was prepared in an analogous manner by cyclization of the appropriate 3-amino-2-pyrazinecarbonitrile (Table II) with guanidine in ethanol.

***N*-[(2,4-Diamino-6-pteridinyl)methyl]-*N*-(3,4-dichlorophenyl)formamide (49, Table IV).** A mixture of 1.7 g (0.0051 mol) of 6-[[(3,4-dichlorophenyl)amino]methyl]-2,4-pteridinediamine (39, Table IV) and 30 mL of 98% formic acid was heated under reflux for 2 h. The residue, after evaporation under a water aspirator, was slurried into 150 mL of hot 85% EtOH and filtered. The filtrate was treated with excess NH_4OH and chilled. The resulting precipitate was collected and dried to give 1.49 g (81%), mp 268–270 °C.

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References and Notes

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(2) This investigation was supported by U.S. Army Medical Research and Development Command Contract DA 17-72-C-2077. This is Contribution No. 1476 to the Army Research Program on Malaria.
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(23) Melting points (corrected) were taken on a Thomas–Hoover capillary melting point apparatus.

Folate Antagonists. 11. Synthesis and Antimalarial Effects of 6-[(Aryloxy- and arylthio-)methyl]-2,4-pteridinediamines and -pteridinediamine 8-Oxides[1-3]

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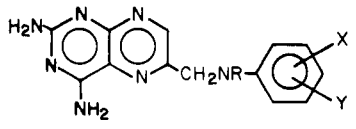
Condensation of 3-amino-6-(bromomethyl)-2-pyrazinecarbonitrile 4-oxide with 4-chlorophenol gave 3-amino-6-[(4-chlorophenoxy)methyl]-2-pyrazinecarbonitrile 4-oxide (1), which was deoxygenated to obtain the de-*N*-oxide 4. Cyclization of 4 and 1 produced 6-[(4-chlorophenoxy)methyl]-2,4-pteridinediamine and the 8-oxide, respectively. 6-[(Arylthio)methyl]-2,4-pteridinediamines and their 8-oxides were produced analogously. Controlled oxidation of the former gave the anticipated sulfoxide 12 and sulfone 13. None of these compounds showed significant activity when tested against lethal *Plasmodium berghei* infections in mice or a select list of bacteria in vitro.

One cannot generally predict the effect that replacement of nitrogen by sulfur or oxygen will have on biological activity. The success achieved with certain of the 6-substituted 2,4-quinazolinediamines,[3] however, warranted preparation of the aryloxy and arylthio analogues of the 6-[(arylamino)methyl]-2,4-pteridinediamines I which had



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been shown to have particularly potent prophylactic effects against *Plasmodium gallinaceum* infections.[1]

Chemistry. As shown in Scheme I, condensation of 3-amino-6-(bromomethyl)-2-pyrazinecarbonitrile 4-oxide[4,5] with 4-chlorophenol in acetone gave 3-amino-6-[(4-chlorophenoxy)methyl]-2-pyrazinecarbonitrile 4-oxide (1) (25% yield). Deoxygenation of 1 with triethyl phosphite gave 3-amino-6-[(4-chlorophenoxy)methyl]-2-pyrazinecarbonitrile (4) (80% yield) (see Table I). Cyclization of 4 and 1 with guanidine then produced 6-[(4-chlorophenoxy)methyl]-2,4-pteridinediamine (10) (40% yield) and the corresponding 8-oxide 7 (52% yield), respectively.

In a similar manner, condensation of 3-amino-6-(bromomethyl)-2-pyrazinecarbonitrile 4-oxide with arylthiols gave the 3-amino-6-[(arylthio)methyl]-2-pyrazinecarbonitrile 4-oxides 2 and 3 (55 and 31% yields, respectively) which on deoxygenation afforded the corresponding 3-amino-6-[(arylthio)methyl]-2-pyrazinecarbonitriles 5 and 6 (85 and 83% yields, respectively). Cyclization with guanidine then produced the desired 6-[(arylthio)methyl]-2,4-pteridinediamines 11 and 14 (87 and 54% yields, respectively) and the corresponding 8-oxides 8 and 9 (83 and 47% yields, respectively).

Treatment of 6-[[(4-chlorophenyl)thio]methyl]-2,4-pteridinediamine (11) with hydrogen peroxide in glacial acetic acid for 1 h at room temperature gave 6-[[(4-chlorophenyl)sulfinyl]methyl]-2,4-pteridinediamine (12) (65% yield). When the oxidation of 11 was allowed to proceed for 17 h, the corresponding 6-[[(4-chlorophenyl)sulfonyl]methyl]-2,4-pteridinediamine (13) was obtained in 61% yield.

Oxidation of the sulfur atom was confirmed by the presence of infrared peaks at 1030 cm^{-1} for the sulfoxide and 1150 and 1310 cm^{-1} in the case of the sulfone.

Suppressive Antimalarial Screening in Mice. The 6-[(aryloxy-, arylthio-, arylsulfinyl-, and arylsulfonyl-)methyl]-2,4-pteridinediamines and *N*-oxides (compounds 7–14, Table II) were tested against a normal drug-sensitive strain of *P. berghei* in mice by the parenteral route.[6,7] The compounds were dissolved or suspended in sesame or peanut oil and were administered to mice in a single subcutaneous dose 72 h postinfection. Extension of the

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